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COMPARATIVE CLINICIAL – HISTOLOGICAL STUDY OF SEMINOGRAMS AND TESTICLE BIOPSIES IN MALE INFERTILITY

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ABSTRACT.

Male infertility represents a problem studied by urologists, pathologists, geneticists, immunologists and researchers from many different disciplines, considering its worldwide growth and the complexity of diagnostics. The authors have indicated the statistical data concerning its extent, especially to the values prescribed by the World Health Organization, and highlighted the various causes of infertility. Special attention has been paid to the importance of precise diagnostics of certain factors which have a share in the development of the discussed issue, which plays a key role in treatment and further prognosis. It has been concluded that control examinations are necessary during the fetal period, throughout the fetal period and after birth, as well as monitoring regarding male infertility factors as a whole, and in the cases of certain disorders it manifests. In this regard, it is necessary to achieve a higher level of health culture among parents, as well as adequate education of professional and scientific workers, in order to recognize the need for control examinations for the purpose of undertaking adequate medical tests in a timely fashion.

Introduction

Fertility implies the ability to reproduce individual organisms. It does not only have a biological, but also a social significance, and is studied in various disciplines, primarily in sociology, demography, public health, etc. Contrary to fertility, infertility represents the incapacity of a sexually active couple that does not use contraception for one year to receive progeny (1). The responsibility for infertility is borne by both partners in different proportions (2, 3). In 2008, the US national statistics reported that 35% of the realized artificial insemination cycles were conditioned by the male factor, 17% by one partner, and 18% by combined diagnoses (4).

Despite the great efforts to discover the "guilty party" of infertility, researchers most often emphasize that approximately 10% of the total registered infertility falls under idiopathic sterility, although a significantly higher proportion is also mentioned (5).

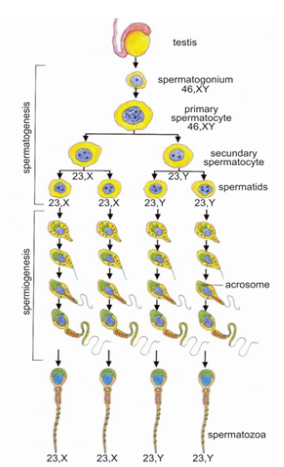
The causes of male infertility are numerous and can be classified into three basic categories: pre-testicular, testicular and post-testicular. For the sake of a better diagnosis, and, therefore, timely therapy, it is essential to possess the knowledge of history and, therefore, knowledge both of the testicles and of the other organs of the male genital tract, as well as the associated glands of the prostate type, seminal vesicles and bulbourethral glands (6, 7, 8, 9). The latter structures play an important role in the formation of the final composition of the ejaculate, allowing the normal function of spermatozoa in the process of fertilization.

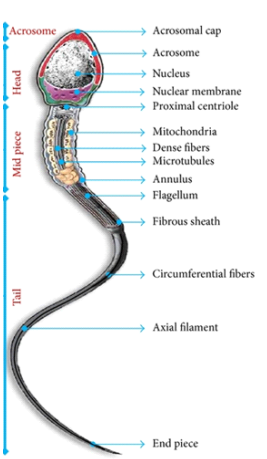
To understand the problem of male infertility, it is necessary to familiarize oneself with the following: the anatomy of the testicle and its role, the endocrine status of the male reproductive system, and the process of spermatogenesis.

In terms of hormonal status, testicular endocrinology is very complex. It consists primarily of FSH (follicle-stimulating) and LH (luteinizing) hormone, formed under the influence of the middle pituitary gland, where their role in creating spermatozoa is of the highest importance. FSH is a glycoprotein whose level in men does not show oscillations as it does in women, while LH glycoprotein is composed of two lower-level units, and plays a role in the stimulation of Leydig cells.

Spermatogenesis is a complex process in which relatively underdeveloped diploid cells (spermatogonia) evolve into haploid spermatozoa (Pictures 1 and 2). This process aims to ensure the transfer of recombinant DNA within half the genome for progeny with the successful transmission of spermatozoa through the female genital tract and transfer of genetic material to the ovum.

The causes of male infertility are determined on the basis of anamnestic data and findings of seminograms, whose parameters (total number and concentration of spermatozoa, mobility, morphology, presence and number of leukocytes, etc.) are compared with those prescribed by the World Health Organization (1987, 1992, 1996). The reference values have been obtained through sample research in a large number of countries, involving a large number of subjects, in which instance boundaries were set within which a value of the observed research parameters should be represented with a probability of 95% (10).





Picture 1. Diagram of the process of spermatogenesis (11)

Picture 1. Diagram of the process of spermatogenesis (11)Picture 2. The appearance of mature spermatozoa (12)

Aim of the Paper

The aim of the paper is to indicate the specificity of the indicators of male infertility registered in our area from the standpoint of biology, embryology and pathohistology.

Material and Methods

For our research, we used the examined samples of men's ejaculate at the Clinic of Gynecology and Obstetrics within the Department for In vitro Fertilization. The obtained material was subjected to the processes of liquefaction, followed by a microscopic analysis: the volume, the number of spermatozoa (living and dead), the motility, the morphology, and the possible presence of other cells were estimated in order to complete the embryological analysis.

We analyzed surgical biopsies of the criptorchidic testes for a clinical diagnosis of "male sterility". The taken surgical biopsies were fixed during 24 h in 10% formaldehyde solution. The treatment of fixed material was performed in the autotechnicon of the Institute of Pathology and Human Polyclinic in Niš (Serbia).

The paraffin sections of 4-micrometer thickness were stained with the following methods: conventional H&E technique for a histopathologic diagnosis of the present process and various histochemical PAS, Veigert and Van Gieson methods, for the detection of the various histological structures, not only in the lumen content, but also inside the basal membranes of the seminiferous ducts.

The Results of the Embryological Research

This segment of the research refers to the comparison of the reference values of individual ejaculate components, primarily spermatozoa, and the results obtained by examining our patients.

Table 1. The parameters of a normal seminogram in current use

Characteristic	Values
Volume	≥2,0 ml
Sperm count – concentration	≥ 20 x 106/ml
Total number of spermatozoa	≥40 x 106
Motility (mobility) Total progressive mobility „a" rapid progressive mobility	≥50% ≥25%
Vitality	≥50%
Morphological normal sperms (%)	≥15%
Leukocytes	<1 x 106/ml

The obtained values indicate different diagnoses, dependent on the mutual numerical features of individual parameters, from normozoospermia, where all the parameters are in accordance with the prescribed values, to the azoospermia, in which the existence of spermatozoa is not recorded.

The initial study in the field of male infertility at the Clinic of Gynecology and Obstetrics in Niš, conducted in the period between 2008 and 2017, provided the following information on the number and share of patients with the absence of spermatozoa in the embryologically analyzed material.

Table 2. The proportion and structure of azoospermic men in the total number of patients examined by age

Year	Examined	With azoospermia	≤30 y.	31-40 y.	41-50 y.	≥51 y.
2008	11	-	-	-	-	-
2009	475	10	3	7	-	-
2010	752	19	6	10	3	-
2011	623	10	3	6	1	-
2012	595	15	4	8	3	-
2013	690	10	1	8	-	1
2014	486	9	2	6	1	-
2015	449	7	1	6	-	-
2016	593	18	4	12	2	-
2017	585	11	3	3	4	1
Total	5259	109	27	66	14	2
Total with azoospermia (%)		100	24,77	60,55	12,84	1,84

The proportion of azoospermic men examined at the Department for In vitro Fertilization of the Clinic for Gynecology and Obstetrics in Niš, in the 9 years of observation, ranged from 1.4% to 3.0%. The patients were observed by their age. It has been established that azoospermia is most often represented in the age range of 31-40.

The very diagnosis of male infertility requires the application of an entire spectrum of differential diagnostic methods, of which the pathohistological diagnosis is highlighted as the most important diagnostic method, in addition to the physical examination, the determination of the hormonal status, and screening for Y-aberrations.

Results of the Pathohistological Research

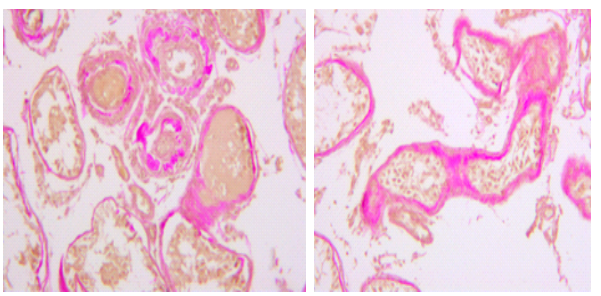
The pathohistological findings obtained through the biopsy of the testicle are one of the most certain possibilities in reproductive practice. This method is used as a factor of choice in men with fully immobile spermatozoa, their abnormal morphology, presence of obstructions, or inexplicable infertility (13). It is therefore understandable that this method is called the "gold standard" in the diagnostics of sterility.

The histopathological analysis reveals various processes, as well as normal testicle build.

Out of the pathological processes, a frequent one is the interruption in spermatozoa maturation to the level of spermatocytes, or the detection of atrophy of the entire testicle, with accompanying hyalinization.

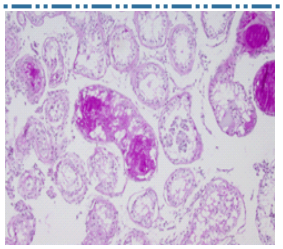
Histopathological findings are also possible, indicating an interruption in the sperm maturation at the spermatocyte level, more severe damage to the testicle in the sense of its atrophy and hyalinization. One of the possible findings of biopsy is the sole presence of Sertoli cells; if it is present on both testicles, it is responsible for azoospermia. Finally, it is also possible that the findings of testicular biopsy performed in infertile men refer to the thickening of the basal membrane of the seminal tubules, although this result cannot be reliably attributed to the cause of infertility.

The prominent results on our material are presented in the following pictures (1-8).

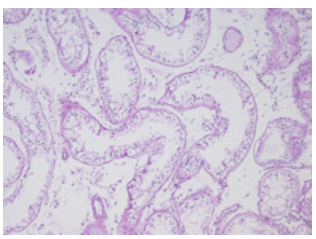


Picture 1. Sertoli cells, with a nodular thickening of the basal membrane. Van Gieson x 200

Picture 2. Sertoli cells with diffuse thickening of the basal membrane. Van Gieson x 200

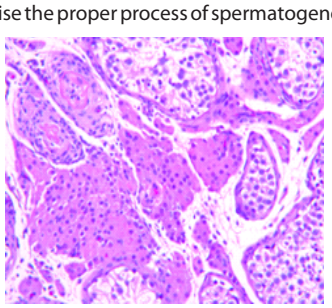


Picture 3. Protein content inside the tubule with germinative cells PAS x 200

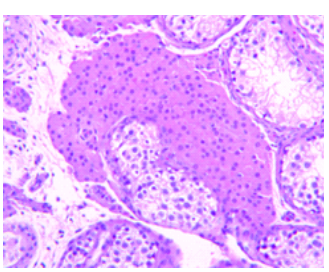


Picture 4. Sertoli cells with clear cytoplasm and without germinative cells. PAS x 200

Pictures 1 to 4 show changes in the Sertoli cells, caused by various factors associated with infertility, such as aging, decreased FSH and LH concentrations, atrophy of the testicles, as well as other factors that compromise the proper process of spermatogenesis.

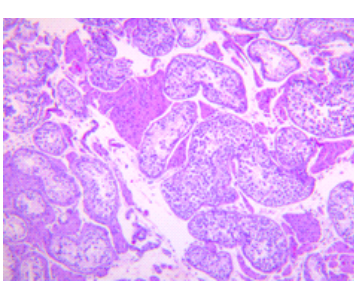


Picture 5. Leydig cell hyperplasia of in the interstitium. PAS &300

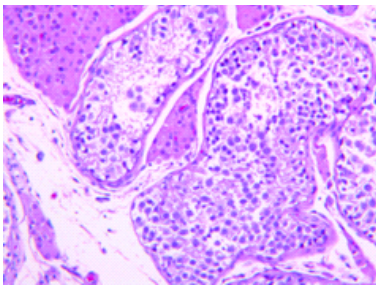


Picture 6. Pink-red cytoplasmic granules in the cytoplasm of Leydig cells. PAS & 300

Pictures 5 and 6 show evident Leydig cell hyperplasia in the interstitium in the case of cryptorchism, as well as in other conditions, primarily the atrophy of the testicle and Klinefelter syndrome.



Picture 7. Reduced diameter of seminiferous tubules, with a decrease in the number of germinative cells. HE x 200



Picture 8. Reduced number of germinative cells, until the complete loss of spermatocytes. HE x 300

In Pictures 7 and 8, the diameter of the seminiferous channels is decreased, with a reduced number of germinative cells.

Discussion

In many samples, unhealthy living conditions damage male fertility. According to Kovač et al., tobacco consumption is reflected in a reduced number of spermatozoa, their motility and abnormalities in morphology, down to the reduced ability for the implantation of embryos (14).

However, while studying the effect of the use of anabolic steroids on infertility, El Osta R noticed anomalies on Leydig cells, apoptosis of germinative cells, necrosis of spermatozoa, cell disomy, and even genetic damage (15).

Studies by Esteves SC have highlighted the importance of a mechanical blockade in obstructive azospermia that occurs along the reproductive tract, including vas deferens, epididymis and ejaculatory ductus. In contrast, in the case of non-obstructive azospermia, the responsible factors are congenital anomalies, post-infectious conditions, gonadotrophin exposure, impact of trauma and endocrine factors (16).

Guč-Šćekić states that genetic factors are responsible for the formation of subfertility and infertility by the deletion on the part of the Y-chromosome, whose function is reflected in the correct regulation of spermatogenesis, which has been called the Azospermia Factor (AZF). The frequency of this phenomenon is estimated at 10 to 15% with the problems of male fertility (17).

Approximately 10% of idiopathic infertility is associated with genetic and epigenetic anomalies, most often due to the translocation of chromosomes, aneuploidy, Y chromosome microdeletion, or gene mutations (18).

In addition to clinical, biochemical and findings reported on the performed biopsies among 1213 infertile men, the latest research (Olesen et al.) has revealed that about two-thirds of the subjects had one or more disorders associated with infertility. These authors cite certain types of biopsies and indications for their application in azoospermia dependent on its cause (19).

Dohle et al. did not reveal the causes of infertility in 30 to 40% of the subjects even with a biopsy analysis (20). Considering that, in certain patients, the localization of sperm obstruction occurs at different levels, it is proposed to use various techniques (21).

Using certain immunohistochemical markers, the status and functions of patients operated on owing to cryptorchism, the presence of spermatogonia and the absence of primary spermatocytes, the fate of Leydig cells, hormonal status, the history of bilateral cryptorchism and fertility potential can now be more closely determined (22).

If the disorder is followed by the hyaline degeneration of the basal membrane with adenomatous hyperplasia of the Leydig cells, the term "seminal tubular hyalinization" is used. In the absence of germ

cells in the tubules, Stouffs K. et al. use the definition: "Sertoly-cell syndrome"(23).

The analysis of testicular biopsies is increasingly used today in the assisted reproduction process when the obtained material of the patients does not contain spermatozoa, or when their number, morphology and motility are insufficient for performing this type of fertilization (24, 25).

Following the above data, the question may arise: is it possible to predict male infertility? Prospective studies in adult men in Denmark and the US, as well as by the WHO, have shown that it is possible to indicate future fertility problems, especially if risk factors are known, such as in the event of cryptorchism or the adoption of unhealthy lifestyles (26).

Conclusion

Male infertility represents a significant medical, biological and socio-psychological problem. The diagnosis is achieved based on the anamnesis, non-invasive methods and testicular biopsy. Its performance is conducted by methods which are conditioned after the differential-diagnostic procedures, and the obtained pathohistological and other results have both a diagnostic and a predictive significance.

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